A COMPARATIVE STUDY OF COLON BACILLI ISOLATED FROM HORSE, COW, AND MAN *

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In the routine analysis of water, to determine its potability, the presence of B. coli in 1 c.c. is taken as an index of pollution. The real danger from a polluted or infected water supply is typhoid fever. The colon bacillus is easily determined if present. The determination of the typhoid bacillus is much more difficult. The typhoid organism is never present in a fecally polluted stream or source of water supply without the colon organism's accompanying it. If the colon bacillus is present, the typhoid bacillus is likely to be present also. It is for this reason that the presence of B. coli is taken as an index of pollution in determining the potability of water.

The colon organism occurs in the intestines of all warm-blooded animals, and is always present in their excrement. Up to the present time, the colon bacilli in the different animals have not been shown to exhibit any marked or distinguishing features. If some feature or features peculiar to the colon bacilli of one animal could be discovered, it might be of material value in water analysis, as indicating the source of fecal pollution.

Acid-forming streptococci, which are also an index of pollution, have been shown by Winslow and Palmer[1] to exhibit marked differences in their ability to ferment carbohydrates. A summary of this work is given in the following table.

TABLE 1

COMPARATIVE FERMENTATION POWERS OF STREPTOCOCCI FROM HORSE, COW, AND MAN

Strain	Percentage of Positive Results (300 Strains) in		
	Lactose	Raffinose	Mannite
Human	62	6	28
Equine	8	4	2
Bovine	52	28	6

* Received for publication January 1, 1916.

[1] Jour. Infect. Dis., 1910, 7, p. 1.

"The rarity of lactose-fermenting streptococci from the horse, makes it probable that this group can be used for distinguishing pollution by street washings from that due to domestic sewage, and the fact that a considerably large proportion of bovine strains ferment raffinose should make it possible to use the ratio to distinguish between washings from pastures and cultivated land from sewage."[2]

Clemesha[3] in India did not obtain these results, however. In addition, the difficulty of isolating the small acid-forming streptococci, in comparison with the ease with which colon bacilli are isolated, is great. The usual media for examining water, litmus lactose agar, would almost entirely eliminate streptococci of equine origin.

With B. coli, the fermentation of carbohydrate media offers no such marked means of differentiation between species or strains of colon bacilli from different animals, altho it serves to break the colon group into separate and distinct organisms.

If a quantitative view were taken in the study of these colon organisms, some good results might accrue and serve as a means of differentiation between the colon bacilli of different origins. The acid-producing properties of these organisms in different carbohydrate media have been worked with, to discover whether the actually measured acid produced, or the quantitative aspect, would offer any means of distinguishing between equine, human, and bovine colon bacilli. The statistical method was first used in this field by Andrews and Horder[4] in 1906 in their work with streptococci. In this country it was first used in 1908 by Winslow and Winslow,[5] in their systematic study of the coccaceae.

Altho the original aim of this work has not been realized, some very interesting and useful information has been obtained through the study.

HISTORICAL REVIEW

The fermentation of the different carbohydrate media and allied substances by the colon group, has been studied with a view to separating the different members of the group and classifying them by means of the fermentation reactions. Büchner[6] in 1885 was the first to note the production of acid and gas in sugar broth inoculated with a colon organism. The real work began with Durham[7] in 1900. It has since been increased and augmented by the work in England of Houston[8] and MacConkey,[9] and in this country by the work of Winslow

[2] Prescott and Winslow: Elements of Water Bacteriology, 1913, p. 210.
[3] Bacteriology of the Surface Waters in the Tropics, 1912.
[4] Lancet, 1906, 171, p. 708.
[5] The Systematic Relation of the Coccaceae, 1908.
[6] Cited by Browne, Jour. Infect. Dis., 1914, 15, p. 580.
[7] Jour. Exper. Med., 1900, 5, p. 353.
[8] Cited by Browne, Jour. Infect. Dis., 1914, 15, p. 580.
[9] Jour. Hyg., 1905, 5, p. 353.

and Walker,[10] Graham Smith,[11] Howe,[12] Jackson,[13] Browne,[14] and Rogers, Clark, and Davis,[15]

Durham[16] on the basis of fermentation (acid and gas in carbohydrate) broke up the intestinal organisms into 3 main groups, (1) B. typhi, which does not ferment dextrose, lactose or saccharose, (2) B. enteritidis, which ferments dextrose, but not lactose, and (3) B. coli, which ferments both dextrose and lactose. This was the starting point. Houston[17] and Savage[18] each tried to distinguish between colon bacilli isolated from different animals, but in this they were unsuccessful.

Winslow and Walker,[19] working with 25 strains of human colon bacilli, found acid produced with dextrose, galactose, lactose, maltose, xylose, and dextrin. None fermented inulin or maltose. With raffinose, saccharose, and dulcite, some caused fermentation while others did not. Smith[20] measured the acid produced in different sugars by B. coli, and Howe's[21] results with 641 strains of coli from 21 men, 540 of which were nonliquefiers, agreed with Smith's in that the sugars formed a metabolic gradient. He came to the conclusion that gas-production was a poor criterion and that acid-production was a sound one. He also broke the organisms up into groups, according to their power of fermenting or not fermenting the test substances. The last step was carried much farther by Jackson,[22] who broke the so-called colon group up into 4 distinct organisms —B. communior, B. communis, B. aerogenes, and B. acidi-lactici—by means of 4 test substances, dextrose, lactose, dulcite, and saccharose. B. communior is dextrose +, lactose +, dulcite +, saccharose +. B. communis is dextrose +, lactose +, dulcite +, saccharose —. B. aerogenes is dextrose +, lactose +, dulcite —, saccharose +. B. acidi-lactici is dextrose +, lactose +, dulcite —, saccharose —.

Rogers and his co-workers,[23] who published papers dealing with the fermentation powers of colon organisms isolated from cows, were especially interested in the gas-production and the relation of the hydrogen to the carbon dioxid. They also measured the acid produced in carbohydrates but drew no definite conclusions from this part of the work. They obtained rather high percentages of acid. None of the 150 strains fermented inulin.

Browne[24] studied several types of human coli, some isolated from polluted waters at Narragansett Bay, R. I., some isolated from Italian immigrants, and some isolated from laboratory assistants. He found that the maximal acid-production took place in 24 hours at the optimal temperature of 37.5 C.; that the most favorable medium contained 1% of the test substance; and that the amount of acid produced was fixed by the organism's toleration of acid. There is an end point for acid-production for each organism. This was confirmed by

[10] Science, 1903, 17, p. 797.
[11] Cited by Browne, Jour. Infect. Dis., 1914, 15, p. 580.
[12] Science, 1912, 35, p. 225.
[13] Jour. Am. Pub. Health Assn., 1911, 1, p. 938.
[14] Jour. Infect. Dis., 1914, 15, p. 580.
[15] Ibid., 1914, 14, p. 411.
[16] Prescott and Winslow: Elements of Water Bacteriology, 1913, p. 93.
[17] Ibid., p. 141.
[18] Ibid.
[19] Science, 1907, 26, p. 797.
[20] Centralbl. f. Bacteriol., 1895, 18, p. 494.
[21] Science, 1912, 35, p. 225.
[22] Jour. Am. Pub. Health Assn., 1911, 1, p. 938.
[23] Jour. Infect. Dis., 1914, 14, p. 411; 15, p. 99.
[24] Ibid., p. 580.

Klígler.[25] If the initial reaction is alkaline, more acid is produced than if the reaction is neutral. Less is produced if the medium is acid.

This in a brief way sums up the work that has been done in regard to the fermentation of carbohydrate media and allied substances by the colon group of organisms.

COLLECTION OF MATERIAL

The bovine and equine colon bacilli used were isolated from feces, the human colon bacilli from raw sewage. One hundred samples of horse manure were collected from the streets of West Lafayette and from the waiting stables in Lafayette. The samples of cow feces were secured from about 10 different farms within a radius of 10 miles of Lafayette. The sewage from which the human strains were isolated was obtained at the opening of the West Lafayette sewer into the Wabash river. Samples were collected on several different days and at different times to insure different strains. As the ground was hard and frozen at the time, neither the street washings nor the leachings from pasture lands got into the sewage; it was all household waste.

ISOLATION OF THE ORGANISMS

In no case were more than 2 strains of colon bacilli isolated from a sample of sewage or from a sample of feces, and usually but one was taken from the sample.

The isolation was accomplished with about the same procedure in each case but with a variety of culture media. A small amount of feces was inoculated into sterile lactose peptone bile (1% lactose, 1% peptone). After 24 hours at 37.5 C. a platinum loopful of this material was plated with litmus lactose agar and again incubated for 24 hours at 37.5 C. Then typical colonies were picked from the plates and streaked on litmus-lactose-agar plates. After 24 hours' growth at 37.5 C., typical colonies were inoculated on agar streaks. These served as the stock cultures for the work.

At times, instead of using lactose peptone bile, broth was used in conjunction with litmus lactose agar. Good results were obtained by substituting endo media in the place of litmus lactose agar, endo media being a lactose agar to which fuchsin has been added, the whole being then decolorized with sodium sulfite.

The action of the bile salts in the bile inhibits the development of the saprophytic bacteria, but does not inhibit either typhoid or colon bacilli. On litmus lactose agar, the colon organisms give rise to typical red colonies, due to the production of lactic acid. With endo media, brick-red colonies develop in the case of colon bacilli, due to the production of lactic acid, which neutralizes the sodium sulfite, thus allowing the fuchsin to regain its color.

TECHNIC

Seven different carbohydrates were worked with. One monosaccharid, dextrose; 2 disaccharids, lactose and saccharose; 1 trisaccharid, raffinose; 1 hexatomic alcohol, mannite; 1 glucosid, salicin; and 1 starch, inulin.

One percent of each of these test substances was added to the sugar-free broth, made according to the standard directions of the American Public Health Association. Three grams of Liebig's extract per liter were used instead of chopped beef. The muscle sugar present in the broth was eliminated by inoculating the sterile infusion of meat extract and distilled water, with a virulent culture of B. coli and incubating at 37.5 C. for 24 hours. This destroyed the

[25] Jour. Infect. Dis., 14, p. 81.

muscle sugar by fermentation. The material was then made into standard broth and sterilized. Usually 7 liters of material were made up at one time.

The amount of broth necessary for one test substance (usually 1 liter) was measured out and 1% of the test substance added. The reaction was adjusted as nearly as possible to the neutral point, phenolphthalein being used as an indicator.

About 8 c.c. of each test medium were run into test tubes, 105 in all, and sterilized. The media were then ready for inoculation.

One set of strains was worked with at a time. The tubes were inoculated with the organisms from a 24-hour-old broth culture, by means of a standard platinum loop. They were then incubated at 37.5 C. for 24 hours; then titrated with N/20 NaOH and the amount of acid produced in each individual case recorded. Controls, prepared at the same time, were tested for sterility and for the initial reaction of the media. Five cubic centimeters of the substance were run into 45 c.c. of distilled water contained in a 150-c.c. Erlenmeyer flask. Phenolphthalein was added and the titration proceeded with.

In addition to the acid-production in the different test substances, a few other characters were studied; e. g., the gram stain, gelatin-liquefaction, and indol-production.

The 100 strains of colon bacilli of each type caused no liquefaction of gelatin within a week, were all gram-negative, and all productive of indol in peptone solution. The results with respect to acid-production by human, bovine, and equine colon bacilli are given in Table 2.

TABLE 2

ACID-PRODUCTION BY COLON BACILLI

PERCENTAGE OF ACID IN TERMS OF NORMAL NaOH

Strain	Dextrose	Mannite	Salicin	Lactose	Saccharose	Raffinose	Inulin
			A. BY HUMAN COLON BACILLI				
1	2.6	2.3	2.0	2.3	1.6	1.2	—
2	2.5	2.7	—*	2.4	—	—	—
3	2.6	2.5	2.5	2.4	2.6	1.7	—
4	2.5	2.5	2.5	2.2	2.3	1.3	—
5	2.4	2.8	2.1	2.2	2.0	2.0	—
6	2.6	2.4	1.6	2.4	2.7	1.5	—
7	2.6	2.4	—	2.8	1.0	1.6	—
8	2.5	2.4	1.6	2.1	—	1.6	—
9	2.7	2.1	—	2.6	2.4	—	—
10	2.8	2.5	3.8	3.1	2.3	1.4	—
11	2.3	2.4	—	2.0	—	1.6	—
12	2.9	2.6	—	2.8	1.1	—	—
13	2.4	2.7	—	2.1	—	1.2	—
14	2.6	2.7	—	2.4	—	—	—
15	2.6	2.5	—	2.1	—	2.2	—
16	2.7	2.5	1.8	2.3	2.6	1.0	—
17	2.6	2.3	2.2	2.5	2.5	2.3	—
18	2.6	2.6	1.9	2.4	2.3	1.2	—
19	2.8	2.6	1.2	2.4	—	—	—
20	2.6	2.6	—	2.4	2.9	1.3	—
21	2.6	2.8	1.8	2.2	—	—	—
22	2.6	2.3	2.6	2.2	2.3	1.7	—
23	2.7	2.4	1.8	2.2	1.4	1.1	—
24	2.4	2.4	—	2.2	1.3	1.2	—
25	2.6	2.3	—	2.0	1.1	1.2	—

* The sign — means no acid produced.

TABLE 2—*Continued*

ACID-PRODUCTION BY COLON BACILLI

PERCENTAGE OF ACID IN TERMS OF NORMAL NaOH

Strain	Dextrose	Mannite	Salicin	Lactose	Saccharose	Raffinose	Inulin
			A. BY HUMAN COLON BACILLI				
26	2.3	2.2	—	2.2	—	—	—
27	2.5	2.1	—	2.0	2.6	1.5	—
28	2.5	2.2	2.0	2.2	2.8	1.7	—
29	2.7	2.3	2.5	2.3	1.7	1.6	—
30	2.3	2.4	1.7	2.3	—	—	—
31	2.6	2.4	2.5	2.3	2.5	1.5	—
32	2.4	2.4	1.5	2.2	2.6	1.5	—
33	2.4	2.2	2.1	2.3	2.2	1.5	—
34	2.7	2.5	2.6	2.3	2.5	1.8	—
35	2.4	2.4	2.7	2.4	1.6	1.6	—
36	2.6	2.7	3.3	2.3	2.7	1.7	—
37	2.5	2.7	1.0	2.4	2.6	1.6	—
38	2.5	2.5	3.5	2.6	1.3	1.2	—
39	2.5	2.6	3.5	2.5	2.5	1.3	—
40	2.8	2.5	1.9	2.6	2.3	1.5	—
41	2.6	2.3	2.6	2.7	1.2	1.4	—
42	2.4	2.5	2.2	2.3	1.7	1.6	—
43	2.8	2.5	2.5	2.1	2.0	1.2	—
44	2.5	2.5	2.1	2.3	2.8	1.5	—
45	3.1	2.5	1.8	2.3	2.6	1.2	—
46	2.7	2.5	1.9	2.4	2.2	1.3	—
47	2.6	2.5	2.6	2.3	2.5	1.4	—
48	2.7	2.5	1.5	2.3	1.3	1.1	—
49	2.4	2.8	—	2.1	1.1	1.1	—
50	2.6	2.7	—	2.1	2.3	1.3	—
51	2.1	1.7	—	1.9	1.8	2.0	—
52	2.8	2.5	2.8	2.7	1.4	1.7	—
53	2.3	2.6	3.0	2.5	2.0	2.3	—
54	2.4	2.3	2.6	3.0	2.5	1.5	—
55	2.4	2.2	2.4	2.6	2.5	2.0	—
56	3.0	2.2	3.0	4.1	2.5	1.9	—
57	2.5	2.3	2.4	2.2	1.9	1.5	—
58	2.1	2.1	2.3	1.8	1.8	2.2	1.1
59	2.4	2.5	2.1	1.8	2.3	2.3	—
60	3.7	2.1	3.7	4.2	4.0	2.2	—
61	2.4	2.4	2.4	2.0	2.3	2.4	1.2
62	3.5	2.1	2.8	2.3	3.5	3.5	—
63	2.4	2.2	2.5	2.2	2.7	2.3	—
64	3.0	2.2	2.1	2.2	2.4	1.7	—
65	2.6	2.2	2.0	2.3	1.1	1.0	—
66	2.6	2.2	—	2.3	1.6	1.6	—
67	2.4	2.2	1.4	2.3	1.4	1.1	—
68	2.6	2.4	2.1	2.1	2.3	1.8	—
69	2.7	2.3	1.9	2.1	2.5	1.7	—
70	2.6	2.4	—	2.4	1.2	1.6	—
71	2.4	2.1	2.3	2.2	2.3	1.8	—
72	2.3	2.9	2.2	2.2	2.1	2.0	—
73	2.5	2.4	2.2	2.1	2.3	1.7	—
74	2.5	2.1	1.9	4.0	2.3	1.9	—
75	2.6	2.0	2.1	2.1	2.4	2.8	—
76	2.4	1.9	2.6	2.7	2.0	2.7	—
77	2.6	2.0	1.9	1.9	2.3	2.1	—
78	2.5	2.1	2.2	2.2	1.7	1.9	—
79	3.8	2.7	3.3	2.7	2.8	1.7	—
80	2.5	2.3	1.3	2.4	2.8	4.0	—
81	2.5	2.4	1.2	2.1	2.3	1.2	—
82	2.5	2.9	3.7	3.4	4.3	2.4	—
83	2.7	2.3	3.3	2.5	2.2	2.0	—
84	2.6	2.5	2.5	2.5	2.1	1.8	—
85	2.6	2.3	1.5	2.3	1.4	1.3	—
86	2.6	2.7	2.2	2.3	2.3	1.3	—
87	2.5	2.4	1.9	2.2	—	—	—
88	2.4	2.0	2.6	2.1	—	—	—
89	2.6	2.4	2.4	2.2	1.5	1.8	—

TABLE 2—*Continued*

ACID-PRODUCTION BY COLON BACILLI

PERCENTAGE OF ACID IN TERMS OF NORMAL NaOH

Strain	Dextrose	Mannite	Salicin	Lactose	Saccharose	Raffinose	Inulin
			A. BY HUMAN COLON BACILLI				
90	2.6	2.3	2.6	2.3	2.6	2.4	—
91	2.8	2.4	3.3	3.4	2.5	2.1	—
92	3.2	2.3	3.6	2.6	2.3	2.4	—
93	2.4	2.3	2.4	2.2	2.5	2.6	—
94	2.3	2.2	2.3	2.0	2.2	2.4	—
95	2.7	2.3	2.5	2.5	2.4	1.9	—
96	2.2	2.3	2.4	2.6	2.1	2.3	—
97	2.6	2.3	2.0	2.8	2.7	2.3	—
98	2.5	2.0	1.8	2.8	2.3	2.6	—
99	2.6	2.2	1.8	2.2	2.6	—	—
100	2.6	2.2	2.3	2.2	1.2	1.7	—
			B. BY BOVINE COLON BACILLI				
1	2.5	2.4	1.6	1.9	—	—	—
2	2.5	2.5	2.4	2.0	1.2	2.1	1.1
3	2.4	2.4	1.8	2.0	1.3	1.7	—
4	2.5	1.9	2.3	2.1	2.4	2.7	1.4
5	2.3	2.4	1.9	1.9	1.5	1.8	1.4
6	2.6	2.5	1.6	1.9	1.8	1.5	1.3
7	2.5	2.5	1.9	1.9	—	1.7	—
8	2.8	2.5	2.1	1.9	1.2	1.6	1.3
9	2.4	2.4	—	2.1	2.4	1.3	—
10	2.6	2.6	—	1.8	1.5	1.9	—
11	2.4	2.4	1.7	2.1	1.2	1.7	—
12	2.8	2.6	1.9	2.0	2.0	2.5	—
13	2.6	2.5	1.6	2.0	1.3	1.8	—
14	2.6	2.3	—	2.1	1.6	1.8	—
15	2.6	2.5	—	2.0	1.2	1.8	—
16	2.5	2.3	2.3	2.0	1.6	1.5	1.4
17	2.8	2.4	2.0	2.0	1.6	1.9	1.0
18	2.8	2.5	2.5	1.9	3.1	1.8	—
19	2.6	2.0	—	2.1	1.7	1.6	—
20	2.4	2.6	1.4	2.1	1.4	2.1	—
21	2.6	2.9	2.2	2.1	2.4	1.8	—
22	2.7	2.9	1.8	2.1	1.4	2.1	—
23	2.6	2.7	—	2.1	—	2.0	—
24	2.7	2.7	—	1.9	1.4	1.8	—
25	2.6	2.1	1.4	1.9	1.6	2.3	—
26	2.4	2.5	1.9	1.9	1.3	1.8	—
27	2.5	2.5	—	1.9	1.4	1.8	—
28	3.0	2.3	2.6	2.4	3.5	2.6	1.4
29	2.6	2.5	1.7	2.0	1.4	1.8	—
30	2.4	2.4	1.8	2.0	—	—	—
31	2.7	2.6	2.0	2.4	2.5	2.4	—
32	3.3	2.5	2.3	2.0	1.4	2.6	—
33	2.7	2.5	—	2.3	—	2.3	—
34	2.6	2.6	1.3	1.7	1.9	—	—
35	2.7	2.4	1.8	2.9	1.4	2.1	—
36	2.8	2.4	—	2.1	—	1.9	—
37	2.6	2.6	2.0	2.2	1.4	—	—
38	2.5	2.7	1.8	1.8	1.3	1.6	—
39	2.7	2.4	2.3	2.0	1.5	2.4	—
40	2.6	2.5	—	1.7	1.7	1.9	—
41	2.8	2.6	1.7	2.1	2.6	2.0	—
42	2.6	2.4	2.2	2.1	1.7	2.0	—
43	2.8	2.5	3.6	2.1	1.7	2.3	—
44	2.7	2.6	—	2.1	1.7	2.0	—
45	2.6	2.4	—	2.1	1.5	2.3	—
46	2.6	2.6	2.4	2.0	1.3	2.6	—
47	2.7	2.3	1.8	2.1	3.0	2.9	—
48	2.5	2.4	2.2	2.1	1.7	2.4	—

TABLE 2—*Continued*

ACID-PRODUCTION BY COLON BACILLI

PERCENTAGE OF ACID IN TERMS OF NORMAL NaOH

Strain	Dextrose	Mannite	Salicin	Lactose	Saccharose	Raffinose	Inulin
			B. BY BOVINE COLON BACILLI				
49	2.7	2.3	1.8	1.9	2.9	1.8	—
50	3.3	2.1	2.1	2.0	—	2.5	—
51	2.4	2.4	—	1.9	1.6	1.9	—
52	2.5	2.4	1.9	1.9	2.3	1.6	—
53	2.6	2.3	2.1	2.0	3.4	2.8	1.2
54	2.5	2.5	2.2	1.8	2.7	2.3	1.3
55	3.5	2.5	2.8	3.0	2.8	1.8	1.6
56	2.4	2.5	4.1	2.2	1.6	2.0	—
57	2.8	2.3	2.8	1.9	3.1	2.1	1.2
58	2.5	2.5	1.9	2.1	1.4	1.9	—
59	2.5	2.5	2.0	1.9	1.4	2.0	—
60	2.5	2.6	2.9	2.1	1.6	2.1	—
61	2.4	2.5	2.6	1.9	2.6	2.4	1.4
62	2.4	2.8	1.7	2.0	—	—	—
63	3.2	2.6	3.7	2.6	3.6	2.0	—
64	2.8	2.3	3.4	2.7	3.3	2.5	1.3
65	3.7	2.4	2.7	2.3	3.7	2.2	1.5
66	2.7	2.8	1.8	1.7	—	—	—
67	2.6	2.5	2.3	1.6	2.5	2.5	—
68	2.4	2.3	—	1.7	1.5	2.0	—
69	2.6	2.4	2.9	1.8	1.3	1.6	1.2
70	3.4	2.2	2.7	3.2	3.2	2.3	1.3
71	2.6	2.3	3.2	2.0	1.7	2.7	1.4
72	3.0	2.3	2.6	1.8	2.1	2.1	—
73	2.5	2.2	2.8	1.6	1.4	2.4	—
74	2.4	2.3	2.0	2.0	2.6	2.3	1.3
75	2.4	2.4	1.8	1.8	1.6	2.0	—
76	2.7	2.6	2.6	1.7	3.2	2.3	1.3
77	2.6	2.3	2.0	2.1	1.3	1.9	—
78	2.5	3.2	1.5	2.3	1.4	2.6	—
79	2.5	2.4	1.5	1.9	1.1	2.1	—
80	2.6	2.4	1.9	1.9	1.2	2.0	—
81	3.5	2.2	2.9	2.1	3.3	2.2	—
82	2.8	2.6	2.9	2.2	2.7	2.1	2.8
83	2.8	2.6	2.0	1.9	1.9	2.6	—
84	2.7	2.5	—	2.2	1.6	1.1	—
85	3.1	2.6	3.1	2.5	2.4	2.9	—
86	2.6	2.2	—	1.9	—	—	—
87	2.6	2.4	1.8	1.9	1.8	1.8	—
88	2.5	2.4	2.7	2.2	2.3	2.2	1.6
89	2.9	2.4	3.5	2.2	3.0	2.2	—
90	3.0	2.3	3.1	2.0	1.8	2.1	—
91	2.6	2.5	1.5	1.9	1.5	2.2	—
92	2.5	2.8	1.4	1.9	1.7	2.0	—
93	2.5	2.5	1.5	2.1	1.5	2.0	—
94	2.5	2.2	1.9	1.8	1.5	2.0	—
95	2.5	2.3	—	2.0	1.5	2.3	—
96	2.9	2.4	2.3	1.9	2.9	2.0	1.3
97	2.7	2.4	1.7	1.9	1.4	2.2	—
98	2.7	2.4	1.4	2.0	1.6	2.0	—
99	2.5	2.2	1.8	2.0	3.0	2.4	—
100	2.6	2.6	2.0	1.9	1.2	2.1	—
			C. BY EQUINE COLON BACILLI				
1	2.6	1.6	2.0	2.1	2.3	1.5	—
2	2.7	2.2	1.4	2.1	1.3	1.7	—
3	2.5	1.9	1.6	2.1	—	1.8	—
4	2.5	2.1	2.1	2.1	2.7	1.5	—
5	2.4	1.9	1.7	2.1	1.3	1.6	—
6	2.7	2.0	—	1.9	2.1	1.4	—
7	2.9	2.1	3.0	2.1	2.3	1.6	—

TABLE 2—*Continued*

ACID-PRODUCTION BY COLON BACILLI

PERCENTAGE OF ACID IN TERMS OF NORMAL NaOH

Strain	Dextrose	Mannite	Salicin	Lactose	Saccharose	Raffinose	Inulin
			C. BY EQUINE COLON BACILLI				
8	2.4	1.9	—	2.0	1.3	1.5	—
9	2.5	2.1	—	2.0	1.0	1.6	—
10	2.5	2.1	2.0	2.1	3.4	1.7	—
11	2.5	2.0	—	2.1	1.3	1.3	—
12	2.7	2.2	1.8	2.1	2.0	1.5	—
13	2.6	2.1	1.8	2.1	—	—	—
14	2.6	2.0	—	2.4	1.2	1.6	—
15	2.5	2.1	—	2.3	1.2	1.5	—
16	2.5	2.3	—	1.9	1.6	1.9	—
17	2.5	2.2	—	2.1	1.3	1.6	—
18	2.2	2.2	—	2.1	2.4	1.5	—
19	2.7	2.1	2.3	2.1	2.5	2.3	—
20	2.6	1.8	2.4	2.1	1.6	2.0	—
21	2.5	2.0	1.7	2.0	1.4	1.8	—
22	2.4	2.0	1.8	2.1	2.1	1.5	—
23	2.5	2.0	1.6	2.1	1.4	1.9	—
24	2.6	2.0	1.5	2.1	1.3	1.8	—
25	2.4	2.2	—	2.3	1.3	1.5	—
26	2.7	1.9	1.5	2.1	1.3	1.7	—
27	2.3	2.2	—	2.1	1.8	1.6	—
28	2.6	2.0	—	2.2	2.5	1.5	—
29	2.4	2.0	—	2.1	1.3	1.5	—
30	2.4	2.0	1.4	2.1	1.2	1.6	—
31	2.4	2.0	—	2.1	1.3	1.7	—
32	2.5	1.8	1.4	2.1	1.2	1.6	—
33	3.0	2.1	3.4	2.5	4.1	1.6	—
34	2.6	2.1	—	2.0	1.3	1.6	—
35	3.1	2.0	3.1	2.4	4.5	1.7	—
36	2.5	2.3	—	2.3	1.2	1.6	—
37	2.4	2.0	1.8	1.9	—	1.6	1.6
38	2.6	2.4	—	2.1	1.3	1.9	—
39	2.9	2.2	1.9	2.1	—	1.2	—
40	2.7	2.0	—	2.2	1.6	1.5	—
41	2.6	2.0	1.8	1.9	2.7	1.6	—
42	2.4	2.1	1.8	2.3	1.3	2.2	—
43	2.7	2.0	—	2.1	—	—	—
44	2.6	2.2	—	1.9	1.1	1.6	—
45	2.7	2.3	1.9	2.1	1.7	1.6	—
46	2.6	2.1	—	1.9	1.2	1.6	—
47	3.1	1.6	2.3	2.0	2.4	2.0	—
48	2.5	2.0	1.8	1.9	2.7	1.7	—
49	2.8	2.1	3.2	2.5	2.7	1.5	—
50	2.4	2.3	—	2.1	1.2	1.7	—
51	3.1	2.1	2.8	2.8	3.8	1.2	—
52	2.4	1.6	2.0	2.0	1.5	1.8	—
53	2.6	2.3	1.9	2.1	1.3	1.6	—
54	2.4	2.2	1.2	2.1	1.0	1.8	—
55	2.6	1.9	1.5	2.3	3.0	1.9	—
56	2.9	1.9	3.4	2.7	2.6	1.8	—
57	2.9	1.0	2.4	2.7	1.9	1.7	1.0
58	3.4	2.2	3.1	2.9	4.2	1.3	—
59	2.6	2.2	1.2	2.1	1.2	1.4	—
60	3.6	2.1	3.6	1.9	3.8	1.8	—
61	2.4	2.0	—	2.0	—	—	—
62	2.6	2.0	1.6	2.1	1.2	2.0	—
63	2.7	2.2	1.4	1.9	1.4	1.4	—
64	2.8	2.3	2.2	2.4	1.6	1.6	—
65	2.4	2.2	1.9	2.1	—	—	—
66	4.4	2.3	3.0	2.6	2.0	1.3	—
67	2.7	2.1	1.7	2.0	3.3	1.7	—
68	2.6	2.2	—	2.1	1.2	1.5	—
69	4.3	2.1	3.0	3.0	4.1	1.6	—
70	2.5	2.0	1.6	2.2	2.3	1.8	—
71	2.7	1.6	—	2.1	1.5	1.8	—

TABLE 2—*Continued*

ACID-PRODUCTION BY COLON BACILLI
PERCENTAGE OF ACID IN TERMS OF NORMAL NaOH

Strain	Dextrose	Mannite	Salicin	Lactose	Saccharose	Raffinose	Inulin
			C. BY EQUINE COLON BACILLI				
72	2.4	2.1	1.2	2.1	—	1.5	—
73	2.4	1.9	1.2	2.0	1.3	1.8	—
74	4.2	2.1	3.3	3.2	4.0	1.4	—
75	3.2	2.0	3.6	2.1	1.4	1.6	—
76	2.7	2.4	1.6	2.0	1.3	1.8	—
77	2.9	2.1	2.6	2.1	4.2	1.2	1.0
78	2.5	2.3	1.7	1.8	1.1	1.3	—
79	2.7	2.4	1.1	2.1	2.4	1.4	—
80	2.6	2.0	2.6	2.0	—	1.5	—
81	3.6	2.4	3.4	2.2	3.9	1.7	—
82	3.0	2.4	—	2.1	3.0	1.9	—
83	2.7	2.3	1.7	2.0	1.2	1.5	—
84	2.6	2.2	—	1.6	—	1.8	—
85	3.1	3.0	1.7	2.1	1.3	2.0	—
86	2.8	1.7	2.2	2.6	2.3	1.5	—
87	4.2	1.8	2.1	2.8	2.9	2.0	—
88	2.6	2.3	1.5	2.2	1.3	1.7	—
89	2.6	2.0	1.4	2.0	1.0	2.0	—
90	2.4	2.1	1.5	2.1	—	—	—
91	2.7	2.3	1.5	2.2	1.2	1.6	—
92	3.3	2.0	1.9	2.1	1.6	1.8	—
93	2.5	2.1	1.8	2.0	1.1	1.2	—
94	2.6	2.1	1.5	2.1	1.3	1.7	—
95	4.0	1.9	3.2	3.6	2.7	2.5	1.1
96	2.8	2.2	1.5	1.9	1.3	1.3	—
97	3.6	2.0	3.8	2.1	4.2	1.5	—
98	1.1	2.3	—	2.1	1.4	—	—
99	3.3	2.1	3.1	2.6	4.4	1.4	—
100	2.7	2.3	1.9	2.0	2.3	1.5	—

THE ANALYSIS OF THE DATA

The foregoing work may be analyzed according to the following topics:

(1) The number of organisms of each type that produce acid from the test substances, that form indol, that do not liquefy gelatin, that are gram-negative.

(2) The average acid-production by each of the 100 strains of colon bacilli of each type.

(3) The relation between the complexity of the substance fermented and the acid produced.

(4) The relation between the average acid-production and the sugars (dextrose, lactose, saccharose, raffinose) attacked.

(5) The number of strains in each type that produce a given amount of acid and their relation to the substance fermented.

(6) The relation between the average amount of acid produced in each test substance and the origin of the organisms.

TABLE 3

PERCENTAGE OF ORGANISMS ATTACKING THE VARIOUS SUBSTANCES

Substance	Human	Bovine	Equine	Average
Dextrose	100	100	100	100
Mannite	100	100	100	100
Lactose	100	100	100	100
Raffinose	89	93	95	92
Saccharose	88	90	91	90
Salicin	82	82	73	79
Inulin	2	24	4	10
Gelatin (—)	100	100	100	100
Gram stain (—)	100	100	100	100
Indol (+)	100	100	100	100

TABLE 4

THE AVERAGE ACID-PRODUCTION IN PERCENTAGE OF NORMAL NaOH

Substance	Human	Bovine	Equine	Average
Dextrose	2.57	2.67	2.74	2.66
Mannite	2.31	2.43	2.08	2.27
Lactose	2.38	2.04	2.13	2.18
Saccharose	2.18	1.95	1 94	2.02
Salicin	2.32	2.16	2.13	2.20
Raffinose	1.76	1.88	1.63	1.76
Inulin	—	—	—	—

TABLE 5

THE RELATION BETWEEN THE AVERAGE AMOUNT OF ACID PRODUCED AND THE SOURCE OF THE ORGANISM

Test Substance	Source	Average Acid-Production
Dextrose	Horse	2.74%
	Cow	2.67
	Man	2.56
Mannite	Cow	2.43%
	Man	2.31
	Horse	2.08
Lactose	Man	2.38%
	Horse	2.13
	Cow	2.04
Saccharose	Man	2.19%
	Cow	1.95
	Horse	1.94
Salicin	Man	2.34%
	Cow	2.15
	Horse	2.13
Raffinose	Cow	1.88%
	Man	1.76
	Horse	1.63
Inulin	Cow	—
	Horse	—
	Man	—

From Table 3 it is evident that all the strains studied give the typical reactions of B. coli with gelatin, with the gram-stain, and also with regard to the production of indol. The different types agree in that all produce acid in dextrose, lactose, and mannite. The outstanding result of the tests with the other sugars is that a number of the strains of bovine origin produce acid in inulin broth.

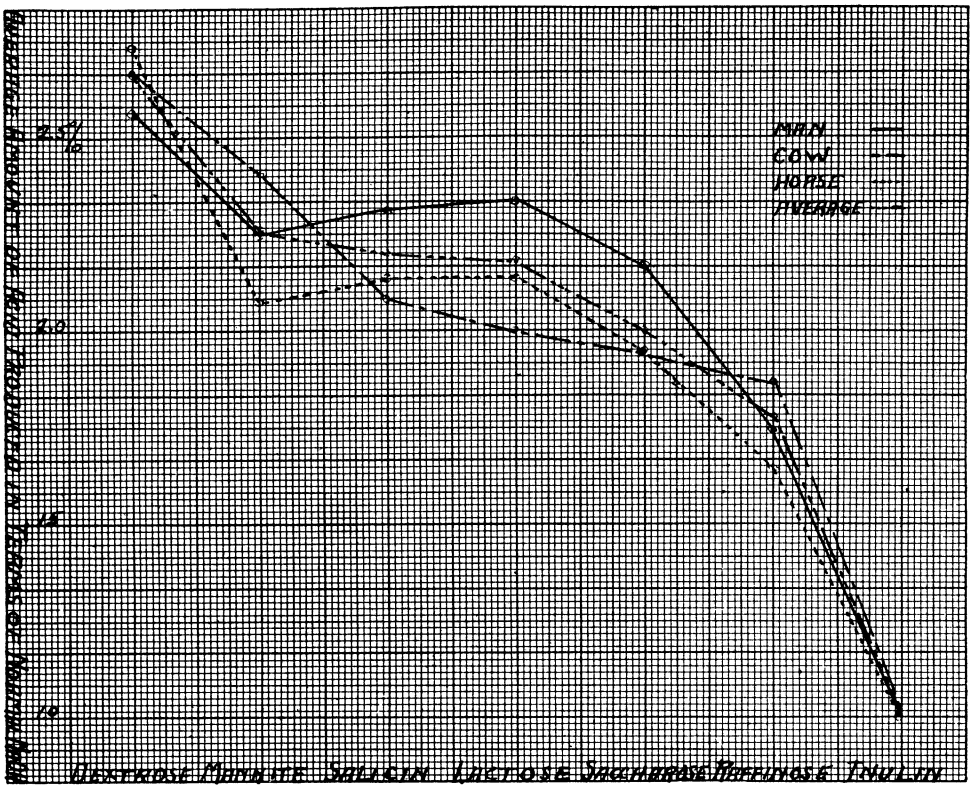

Chart 1. Average acid-production in the test substances.

In general, according to these data, the average amount of acid produced in the different test substances varies from 2.67% to 1.63%. The greater amount of acid is usually produced in substances of lesser complexity. For example, on the average there is more acid produced in dextrose, less in mannite, less in lactose, less in saccharose, less in raffinose, and less in inulin. Salicin and mannite occupy a variable position. When considering the sugars alone—dextrose, lactose,

saccharose, and raffinose—a true metabolic gradient is formed in each of the 3 types and also in the average of the 300 strains taken together. These relations are shown in Charts 1 and 2.

From a perusal of the data it is seen that the acid-production, for the greater part, lies within narrow limits in the case of dextrose, lactose, and mannite. These substances were fermented by every

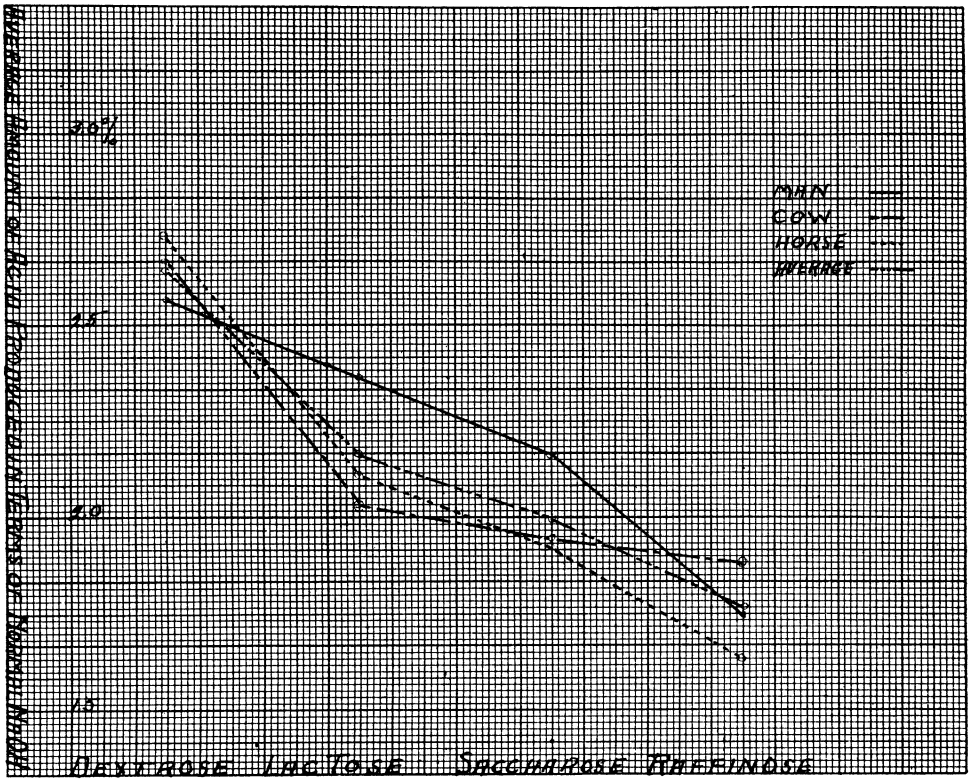

Chart 2. Average acid-production in the sugars.

strain. The larger number of strains in each case produce a certain amount of acid, and the others lie near this point.

With raffinose and saccharose acid-production is not, generally speaking, confined to narrow limits but is spread out. In the case of salicin curves, the acid-production is spread over a very large area.

In the case of dextrose, lactose, and mannite, the high points of acid-production lie close together. In the case of dextrose the high

point of acid-production was the same with human, bovine, and equine colon bacilli, namely, 2.6%; in the case of mannite, it was, human, 2.4%, bovine, 2.4% and equine 2.1%; in the case of lactose it was, human, 2.3%, bovine, 1.9%, and equine, 2.1%.

From the data, it is evident that the average percentages of acid produced by the 3 different types of colon bacilli, do not lie far apart in the case of any given test substance.

It is also evident that the average acid-production does not serve to differentiate between the strains of colon bacilli. For example, in one case bovine colon bacilli head the list, in another case they are at the bottom, in another in the middle. This is also true of the other two types.

CONCLUSIONS

On an average, the different types of strains—human, bovine, and equine—exhibit a remarkable similarity in all reactions tested, chiefly in acid-production. One remarkable exception was the ability of 24 strains of bovine colon bacilli to produce acid in inulin media. The other differences were not marked enough to be of value.

In all cases the average acid-production for each of the 100 strains of each type resembles that of every other one, and also resembles the average acid-production of all the strains taken together irrespective of origin.

In general, the average amount of acid produced by each type tends to decrease as the complexity of the test substance increases.

A metabolic gradient is definitely shown with the sugars, dextrose, lactose, saccharose, and raffinose. It is clearly shown in each case and also in the average of the three types taken together.

The organisms show a preference for some of the test substances. For example, the average for all the organisms shows that 100% act upon dextrose, lactose, and mannite; 90% on saccharose; 92% upon raffinose; 79% upon salicin; and 10% upon inulin.

With mannite, dextrose, and lactose, the organisms have a high point of acid-production at which the larger percentage of the strains belong. The other strains for the greater part lie immediately on either side of this high point. The acid-production for the larger number is confined to narrow limits.

The high points of acid-production do not lie far apart with dextrose, lactose, and mannite. They coincide in the case of mannite.

In general with saccharose, raffinose, and salicin, this high point is neither clearly shown nor definitely marked. The acid-production varies greatly and is spread over a large area.